REVIEW ARTICLE

Getting the glycosylation right: Implications for the biotechnology industry

Nigel Jenkins*, Raj B. Parekh[1], and David C. James[2]

*Department of Biological Sciences, De Montfort University, Leicester LE1 9BH, UK. [1]Oxford Glycosciences, Hitching Court, Blacklands Way, Abingdon OX14 1RG, UK. [2]Research School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK. *Corresponding author (e-mail: njenkins@dmu.ac.uk).*

Received 4 March 1996; accepted 18 June 1996.

Glycosylation is the most extensive of all the posttranslational modifications, and has important functions in the secretion, antigenicity and clearance of glycoproteins. In recent years major advances have been made in the cloning of glycosyltransferase enzymes, in understanding the varied biological functions of carbohydrates, and in the accurate analysis of glycoprotein heterogeneity. In this review we discuss the impact of these advances on the choice of a recombinant host cell line, in optimizing cell culture processes, and in choosing the appropriate level of glycosylation analysis for each stage of product development.

Keywords: glycosylation, recombinant, antigenicity, clearance, review, carbohydrates

A better understanding of glycoprotein biosynthetic pathways and cloning of many of the key enzymes involved, together with progress in assigning functions to specific carbohydrate structures, has provided the groundwork for exploitation of glycobiology within the biotechnology industry. The carbohydrate components of glycoproteins can play crucial roles in protein folding, oligomer assembly and secretion processes, and in the clearance of glycoproteins from the bloodstream. Certain carbohydrate structures have also been found to be antigenic, and regulatory authorities such as the US Food and Drug Administration are demanding increasingly sophisticated carbohydrate analyses as part of the product or process validation[1,2].

It is not the purpose of this review to provide a general update on advances in glycobiology, which have already been covered by several excellent reviews published on biosynthetic pathways[3-6], the biological properties of carbohydrates[7], and cellular influences on the glycosylation process[8-11]. Instead, this review will focus on the differences in carbohydrate structures that may arise from choosing alternative gene expression systems and culture conditions, their physiological significance, and the level of carbohydrate analysis that is appropriate at each stage of product development.

Oligosaccharide structures found on glycoproteins

Oligosaccharides can attach to proteins in three ways (Fig.1): (1) Via an *N*-glycosidic bond to the *R*-group of an Asn residue within the consensus sequence Asn-*X*-Ser/Thr (*N*-glycosylation). All mature *N*-linked glycan structures have a common core of $Man_3GlcNAc_2$ which can form part of simple oligomannose structures or be extensively modified by the addition of other residues such as fucose, galactose, and sialic acids. Hybrid structures also exist where one or more arms of the glycan are modified and the remaining arm(s) contain only mannose. (2) Via an *O*-glycosidic bond to the *R*-group of Ser or Thr (*O*-glycosylation). *O*-linked glycosylation is extensive in structural proteins such as proteoglycans. Small glycan structures can also be *O*-linked to the side chain of hydroxylysine or hydroxyproline. (3) Carbohydrates are also components of the glycophosphatidylinositol anchor used to secure some proteins to cell membranes.

The presence of these consensus sequences by no means guarantees their glycosylation. They show varying degrees of occupancy with oligosaccharides (macroheterogeneity) dependent on their position within the protein and its conformation, the host cell type used for expression, and its physiological status. These three factors also determine the extent of variation in the type of sugar residues found within each oligosaccharide (microheterogeneity).

Choice of expression system

Bacteria. Common bacterial expression systems such as *Escherichia coli* have no capacity to glycosylate proteins in either *N*- or *O*-linked conformations[12]. Although other bacterial strains such as *Neisseria meningitidis* have recently been shown to *O*-glycosylate certain of their endogenous proteins, the trisaccharide added is different from *O*-linked sugars found in eukaryotes[13].

Yeast. Hypermannosylation (the addition of a large number of mannose residues to the core oligosaccharide), is a common property of most yeast strains[14] and can compromise the efficacy of recombinant proteins such as the hepatitis B vaccine[15] (Table 1). But hypermannosylation can be prevented by expressing the polypeptide in mutant yeast strains (e.g., mnn-9 or the temperature-sensitive ngd-29) in which *N*-glycosylation is confined to core oligosaccharide residues with a limited mannose content (up to $Man_5GlcNAc_2$), resulting in more effective vaccines[15,16]. There is also evidence to suggest that different *O*-glycosylation sites are used by yeast and mammalian cells[17].

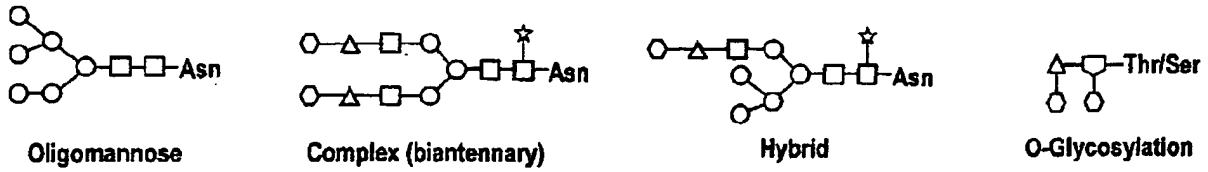


Figure 1. Common oligosaccharide structures found on glycoproteins: ○ Man, □ GlcNAc, △ Gal, ◇ NeuAc, ★ Fucose, ▽ GalNAc.

Plants. The few studies reporting the production of human proteins in plants have suggested that simple *N*-glycan structures that lack sialic acids are added, which may compromise activity. For example, erythropoietin (EPO) produced in tobacco cells has no biological activity in vivo, presumably because of its high clearance rate[14]. Another obstacle may be the presence of potentially allergenic residues such as core α1-3 linked fucose[15] or xylose β1-2 mannose[16].

Insects. The baculovirus-infected insect cell expression system has become a popular route for recombinant protein synthesis because of its short process development time and potentially high yields. Most evidence to date indicates that the *N*-glycosylation capabilities of this system are limited to producing only simple oligomannose-type oligosaccharides ($Man_{1-3}GlcNAc_2$)[11-13]; only a few studies have demonstrated complex *N*-linked glycans[14]. However, most of these data are derived from *Spodoptera frugiperda* (Sf9 and Sf21 lines), and other baculovirus-infected insect cell lines may differ. For example, the Ea4 line (derived from *Estigmena acrea*) can add some terminal galactose residues to recombinant interferon-γ[15], and secreted alkaline phosphatase produced in *Trichoplusia ni* (TN-368 and BTI-Tn-5B1-4 lines) has both galactose and terminal sialic acids[16].

Mammals. Species that are phylogenetically closer to humans may be expected to have more elements of the glycosylation machinery in common. Nevertheless, there are some surprising differences between the glycosylation characteristics of the rodent cell lines (routinely used for recombinant glycoprotein synthesis) and human tissues.

Mouse cells. Most mammals express the enzyme α1–3-galactosyltransferase, which generates Galα1,3-Galβ1,4-GlcNAc residues on membrane and secreted glycoproteins. The notable exceptions are in humans, apes, and Old World monkeys where the gene has become inactivated through frameshift mutations[17]. Certain mouse lines such as hybridomas, mouse-human heterohybridomas, and C127 cells synthesize some glycans terminating in Galα1,3-Galβ1,4-GlcNAc[18,19], particularly when grown in nonagitated flasks[20,21]. But other rodent lines such as mouse NS0 or rat Y0 mycloma, producing humanized antibodies, do not add Galα1,3 Galβ1,4-GlcNAc[12,13], and only induce a mild human immune response[14]. These residues are more likely to occur in hybridoma-derived antibodies where glycosylation occurs in the light chain, compared to the partially buried Fc glycosylation site[15]. Over 1% of

Table 1. Summary of the major glycosylation attributes for different cell expression systems.

			Type of Glycosylation			Saccharide Residues								
						Fucose		Galactose		Sialic acids				
rganism	Cell type	O-linked	Oligo-mannose	Hyper-mannose	Complex	α1-6 linked	α1-3 linked	Gal α1-3 Gal	SO_4-GalNAc	α2,6 linked	α2,3 linked	NeuGc	Bisecting GlcNAc	Glyco-sidases
Bacterium	*E. coli*	0	0	0	0	0	0	0	0	0	0	0	0	?
Yeast	*Sacromyces*	++	0	++++	0	0	0	0	0	0	0	0	0	?
Plant	Tobacco BY2	?	++	0	?	?	+-	?	0	0	0	0	0	?
Insect	*S. frugiperda* Sf9	-+	++++	0	D	++	+	0	0	0	0	0	0	++
	S. frugiperda Sf21	-+	++++	0	D	++	?	?	?	D	D	?	0	?
	Trichoplusia ni	?	++	0	++	?	?	?	?	++	++	?	0	?
	E. acrea Ea4	?	+++	0	++	++	0	0	0	0	0	0	0	?
	M. brassicae	++	++++	0	0	++	?	?	?	?	?	?	0	?
Hamster	BHK	++	++	0	++	++	0	+	0	0	++	?	0	?
	CHO	++	++	0	++	++	D	0	0	0	++	+	0	++
Mouse	Hybridoma	++	++	0	++	++	0	++	0	0	++	+++	0	+
	Myeloma	++	++	0	++	++	0	++	0	+	+	+++	0	+
	C127	++	++	0	++	++	0	++	++	++	++	+++	0	?
	J558L	++	?	0	++	++	0	++	?	++	++	+++	0	?
	Transgenic	++	++	0	++	++	0	?	?	+	+	?	?	?
Rat	Y0 Myeloma	?	?	0	++	?	0	?	?	+	+	?	++	?
Goat & Sheep	Transgenic	?	++	0	++	++	0	?	?	+	+	?	?	?
Human	Liver	++	+	0	++	++	0	0	0	++	++	0	0	?
	Brain	++	++	0	++	++	++	0	0	++	++	0	+	?
	Pituitary	++	++	0	++	++	0	0	+++	+	+	0	++	?
	B-lymphocyte	++	0	0	+	++	0	0	0	+	+	0	-+	?
	Namalwa	++	++	0	++	++	0	0	0	++	++	?	?	?
Human-Mouse	Heterohybridoma	?	++	0	++	++	0	0	0	+	+	÷	0	?

Note that a few studies have analyzed the glycosylation profile of the same glycoprotein expressed in more than one system, therefore some characteristics may be protein-specific. Further details of individual cell lines are given in the text. 0=not detected; ?=not tested; D=disputed (conflicting results reported in different publications). + to +++-: an approximation of the levels of oligosaccharides detected.

human serum IgG is directed against the Gal(α1,3)-Gal-β1,4-GlcNAc epitope[36], which may a be consequence of its presence on enteric bacteria. Specific removal of this epitope from porcine endothelial cells substantially diminishes their reaction with the natural cytotoxic antibodies found in human serum[37], but comparisons of mouse C127 and chinese hamster ovary (CHO)-derived tissue plasminogen activator (tPA) reveal only minor differences in the pharmacokinetics induced by interaction with these antibodies[38].

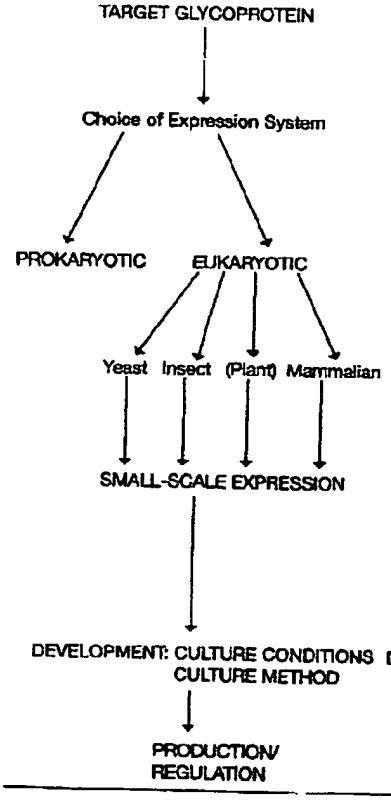
N-glycolylneuraminic acid (NeuGc) is a derivative of the sialic acid *N*-acetylneuraminic acid (NeuAc), and NeuGc levels have been shown to be more prevalent than NeuAc in antibodies derived from mouse or human-mouse hybridomas[39]. In contrast, glycoproteins in adult humans do not normally contain NeuGc, which is an oncofetal antigen[39]. Low levels of NeuGc (1% of total sialic acids) are tolerated in recombinant proteins such as EPO, but higher levels (e.g., in fetuin containing 7% NeuGc) can elicit an anti-NeuGc immune response[40]. Furthermore, high levels of terminal NeuGc on a chimeric CT4-IgG fusion protein are correlated with a rapid removal of the molecule from circulation, compared to the same protein bearing terminal NeuAc residues[41]. The hydroxylase enzyme that converts cytidine 5′-phosphate (CMP)-NeuAc to CMP-NeuGc has recently been cloned[42].

Hamster cells. Most of the CHO cell lines used for recombinant protein synthesis, such as Dux-B11, have fortuitously inactivated the gene for α1-3-galactosyltransferase[43] and make low levels of NeuGc (NeuAc is the dominant sialic acid found on CHO-derived glycoproteins)[44]. But cell lines such as CHO and baby hamster kidney (BHK) lack a functional α2,6-sialyltransferase (ST) enzyme, and synthesize exclusively α2,3-linked terminal sialic acids via α2,3-ST, in contrast to human and mouse cells, which have both enzymes[45]. Most rodent cells, including the CHO cell line, can be genetically modified to resemble the human glycan profile by transfection of appropriate glycosyltransferases. For example, the gene for rat α2,6-ST has been cloned into a recombinant CHO cell line to produce glycoproteins with both α2,6-linked and α2,3-linked NeuAc[45–47]. In addition, many mutants of CHO cells that display altered glycosylation properties that may prove useful hosts for expressing glycoproteins with minimal heterogeneity have been isolated[48].

Human cells. Other host cell lines have not been studied in sufficient depth to precisely define their glycosylation capabilities. Even the use of human host cell lines is not perfect, since the transformation event required in most cases to produce a stable cell line may itself result in altered glycosylation profiles[49]. However, the human lymphoblastoid Namalwa cell line performs *O*-linked and *N*-linked glycosylation efficiently, and preliminary studies on Namalwa-derived recombinant tPA have demonstrated all the human-type glycosylation characteristics[50,51]. All mouse-human heterohybridomas examined thus far have been found to follow the glycosylation characteristics of the mouse parental line[34,52].

Transgenic animals. Relatively few studies have been reported on the glycosylation of recombinant proteins expressed in the milk of transgenic animals. Those published from experiments in transgenic goats[53] suggest that a low level of complex glycans may be achieved. Furthermore, interferon-γ (IFN-γ) expressed in transgenic mice showed a greater proportion of truncated and oligomannose structures at the Asn_{97} site compared to IFN-γ that

Table 2. Decisions governing the type of glycosylation analyses appropriate at each stage of production.



	Question	Monosaccharide composition analysis	Glycoform 'fingerprinting'	Structural analysis/ epitope determination
DECISION 1 — Q1.1 Is glycosylation necessary for the therapeutic profile of the target glycoprotein?	Q1.1	—	—	—
DECISION 1 — Q1.2 If glycosylation does not occur, will underlying peptide regions be 'revealed' to the immune system?	Q1.2	—	—	—
DECISION 2 — Q2.1 Are specific glycan structures required for full therapeutic activity?	Q2.1	✓	✓	✓
DECISION 2 — Q2.2 Are certain glycan structures/epitopes to be avoided?	Q2.2	✓	✓	✓
DECISION 2 — Q2.3 Is mammalian glycosylation preferred?	Q2.3	✓	✓	✓
DECISION 3 — Q3.1 Is the anticipated glycosylation pattern obtained in practice?	Q3.1	—	✓	(✓)
DECISION 3 — Q3.2 Is the glycosylation pattern obtained consistent with activity measurements?	Q3.2	—	—	—
DECISION 3 — Q3.3 Is the glycosylation stable in the chosen formulation?	Q3.3	✓	✓	—
DECISION 4 — Q4.1 Can a reproducible glycosylation pattern be obtained on a lot-to-lot basis?	Q4.1	✓	✓	(✓)
DECISION 4 — Q4.2 What is the optimum method to monitor glycosylation?	Q4.2	✓	✓	(✓)
DECISION 4 — Q4.3 What methods/levels of analysis will be submitted to regulatory authorities?	Q4.3	✓	✓	✓
Lot-to-lot analysis on an on-going basis		✓	✓	—

expressed in CHO cells, although the level of glycosylation site occupancy was increased[21]. Glycoproteins can be remodelled in situ by the transgenic expression of extra glycosyltransferases in the mouse mammary gland[24].

Cells derived from specialized tissues. A significant proportion of IgG molecules produced by human B-lymphocytes possess a bisecting GlcNAc residue β1-4 linked to the central β-linked mannose of the core glycan. This residue may play a role in antibody-dependent cell-mediated cytotoxicity, and only certain rodent cell lines such as the rat Y0 myeloma (but not CHO cells or NS0 myelomas) produce recombinant antibodies containing this bisecting residue[25]. The GlcNAc transferase III enzyme which adds the residue has been cloned, thus, the opportunity exists for remodelling chimeric antibodies by transfecting this gene into host cells[25].

A large body of evidence suggests that natural IgG molecules lacking galactose are associated with rheumatoid arthritis[26]. This has also been tested experimentally by presenting the agalactosyl glycoforms of autoantibodies recognizing type II collagen in T-cell–primed mice, resulting in acute synovitis[27]. Thus, expression systems that result in a large proportion of recombinant therapeutic antibodies lacking galactose should be avoided. Low levels of sialic acids are typically found in both recombinant and natural antibodies[23], probably due to steric hindrance at the Fc glycosylation site.

Sulfated residues (SO_4-GalNAc-GlcNAc) appear on the outer arms of glycans attached to several pituitary glycoprotein hormones such as lutcinizing hormone (LH), thyrotropin, proopiomelanocortin and certain proteases such as urokinase[28,29]. A specific GalNAc transferase and a terminal GalNAc sulfotransferase recognize special protein motifs in the nascent peptide (e.g., Pro-Leu-Arg) and are mainly restricted to the anterior pituitary gland. Therefore, only cell lines derived from the pituitary gland or endothelium (such as At20 and 293 cells) are able to perform this sulfation[30,31]. A hepatic receptor binds oligosaccharides terminating with SO_4-GalNAc residues, and could account for the rapid removal of sulfated LH from the circulation in contrast to the removal of follicle-stimulating hormone and chorionic gonadotropin, which bear terminal NeuAc residues[62]. Mouse C127 cells produce a different pattern of sulfation (NeuAc-α3SO_4-6Gal), which occurs after the addition of NeuAc[63].

The clearance of a given glycoprotein from the blood stream is highly dependent on its oligosaccharides, particularly those that are situated on the outer arms of the glycan structures[41]. Indeed, artificial glycosylation sites have even been introduced into small peptides in order to improve their pharmacokinetic properties or to render them resistant to blood proteases[64,65]. There are several receptors for specific oligosaccharide structures that contribute to the clearance of glycoproteins from the bloodstream, the most significant being the asialoglycoprotein receptor[66]. Other binding proteins recognize specific structures such as the Man-α6Man-β4GlcNAc-β4GlcNAc found in interleukin-6 (IL-6)[67], but their significance in the clearance of the majority of glycoproteins remains to be determined[68]. Studies in rats using EPO fractions enriched for particular glycoforms indicate that more highly branched glycans (e.g., triantennary or tetraantennary) are less susceptible to renal clearance than biantennary structures[69].

Cell culture conditions

Once the host cell line has been chosen, the cell culture conditions should be optimized in order to minimize glycoprotein heterogeneity and prevent deterioration of product quality during fermentation. Pioneering studies analyzing glycoproteins from batch samples[70,71] demonstrated that the culture environment can influence both the macroheterogeneity and microheterogeneity of oligosaccharides in recombinant glycoproteins[14,11-21].

Cell culture media. Monoclonal IgG, produced by mouse hybridomas in serum-free medium has higher levels of terminal sialic acid and galactose residues relative to that produced using serum[72], but antibody galactosylation is better in serum-containing medium for recombinant CHO cells[53]. Production of antibodies in cell culture results in more consistent glycosylation than achieved in ascites fluid[51,72,73]. Adaptation of BHK-21 cells producing a recombinant IL-2 mutant, from serum-containing to serum-free medium, results in substantial changes to its glycosylation, such as the complexity of glycan chains (number of arms, and higher levels of terminal sialylation and proximal α1-6 fucosylation), and increases the overall level of glycosylation[74].

The ambient glucose concentration affects the degree of glycosylation of monoclonal antibodies produced by human hybridomas in batch culture[75], and of IFN-γ produced by CHO cells in continuous culture[76,77]. Lipids such as dolichol act as key carriers in the glycosylation process, and lipid supplements alone or in combination with lipoprotein carriers can improve the *N*-glycosylation site occupancy of IFN-γ[78,79]. Work on hepatocytes suggests that provision of cytidine and uridine can also alter protein glycosylation by increasing the availability of nucleotide-sugars[80].

The application of sophisticated analytical techniques to analyze glycosylation microheterogeneity has led to some interesting findings. An increase in the percentage of oligomannose (predominantly $Man_5GlcNAc_2$) and truncated structures has been observed when monitoring both recombinant antibodies made by NS0 myelomas[12,81] and IFN-γ made by recombinant CHO cells[82]. In the former study, supplemental nutrients in fed-batch culture did not prevent this deterioration in glycosylation. In perfusion culture of BHK-21 cells producing a recombinant IL-2 mutant, nutrient limitations (glucose, amino acids, dO_2) led to short-term changes in macroheterogeneity, but microheterogeneity was largely unchanged[83].

Cell status. Lowering the protein synthetic rate by cycloheximide improves the glycosylation site occupancy of recombinant prolactin produced by C127 cells[84], but studies on tPA synthesis in CHO cells suggest that the rate of protein synthesis by itself has little influence on protein glycosylation[85]. Folding and disulfide bond formation certainly can influence the efficiency of *N*-linked glycosylation in some proteins. For example, low concentrations of the reducing agent dithiothreitol prevent cotranslational disulfide bond formation in the endoplasmic reticulum and lead to complete glycosylation of a tPA sequon that normally undergoes variable glycosylation[85]. Cell growth rate influences glycosyltransferase V levels in HepG2 cells[87]. Sodium butyrate is sometimes used to improve protein synthesis, but can change glycosylation by inducing a GlcNAc-transferase involved in *O*-glycosylation[88] and increasing sialyltransferase activity in recombinant CHO cells[89,90]. (Curiously, butyrate has the opposite effect in HepG2 cells[91].)

Bioreactor configuration. Mild hypoxia has minimal effects on the glycosylation of tPA produced by recombinant CHO cells[92], but influences the level of sialylation of recombinant FSH[93]. Similarly, pH changes within the range 6.9–8.2 in the cell culture medium do not have a dramatic effect on the glycosylation profile of recombinant placental lactogen expressed in CHO cells, however there was some evidence for underglycosylation outside this range[95].

Increases in the concentration of ammonium ion in the culture medium above 2 mM may compromise sialyltransferases present in the Golgi, resulting in reduced α2,6-linked sialic acids in G-CSF produced by recombinant CHO cells[94]. Increased ammonium ions also reduced the extent of recombinant placental lactogen *N*-glycosylation by CHO cells, but this was dependent on the pH[95].

Product degradation. Various glycosidase activities have been measured in CHO cell lysates and culture supernatants, the most active of which is sialidase at neutral pH[96,97]. The soluble enzyme has been purified from culture supernatant of CHO cells[98] and can degrade glycans from proteins such as recombinant gp120. The CHO cell gene coding for this soluble sialidase was subsequently cloned and showed structural similarities to bacterial sialidases[99]. Although CHO cells also produce an α-*L*-fucosidase, this enzyme is incapable of releasing core α1,6-fucose from intact recombinant glycoproteins (gp120 or CD4), or the more peripheral Fuc-α1,3-GlcNAc linkage from serum α,-acid glycoprotein[100]. Sialidase, β-galactosidase, β-hexosaminidase, and fucosidase can be detected at low levels in supernatants from mouse 293, NS0, and hybridoma cells, and the sialidase activity is much lower than that found in CHO cells[101].

In a contrasting study, purified monoclonal antibody was incubated with supernatants from various mammalian cell lines (CHO K1, BHK-21, mouse C127, P3-X63. Ag8.653, and a human-mouse heterohybridoma) and an insect cell line (SF-21AE), and only the insect line showed evidence of glycosidase activity in the supernatant[102].

Controlled carbohydrate modification. Advances are being made in the chemical production of oligosaccharides (e.g., synthesis of the core *N*-glycan structure $Man_3GlcNAc_2$ and oligomannose glycans from monosaccharides has recently been accomplished[103,104]). This opens the possibility of adding defined glycan structures after recombinant protein synthesis and secretion. In addition, the cloning and expression in *E. coli* of several key glycosyltransferases will facilitate the postharvest remodelling of glycoproteins produced in cell culture[105,106], making them more acceptable as human therapeutic proteins.

Conclusions

Improvements in glycosylation analysis have enabled scientists to judge how the glycan structures of recombinant glycoproteins compare to their natural human counterparts. Ultimately, production may go beyond the reiteration of the human glycosylation profile, because it may be desirable to alter the product's bioactivity or pharmacokinetics in vivo by altering specific glycan structures. The initial choice of expression system will continue to be of crucial importance, and as more recombinant proteins are expressed in different cell lines a pattern of glycoform predictions can be assembled (Table 1), although these conclusions must remain speculative until more data are available. The influences of the cell culture process (including the effects of scale-up) are not as well defined at present, but as more studies are published generic protocols may emerge to produce consistent (albeit heterogeneous) glycosylation patterns. A more difficult objective for cell technology will be to produce a single invariant protein glycoform, rather than the current mixture of glycoforms each bearing different characteristics (analogous to chiral separation of small molecule drugs).

An increased awareness of the importance of product glycosylation has lead to more detailed carbohydrate analysis at earlier stages of product development than in the past. The choice of a particular cell expression system, and the influence of a different manufacturing process on the biochemical consistency of the product are now being evaluated (Table 2). A small but increasing number of glycosylation analyses are requested by legal departments, who may use the resulting information in patent applications to substantiate claims and to distinguish their organization's material from that of their competitors.

Acknowledgments

NJ and DCJ are supported by grants from the Biotechnology and Biological Research Council of the UK.

1. Geisow, M.J. 1992. Glycoprotein glycans—roles and controls. *Trends Biotechnol.* 10:333–335.
2. Liu, D.T.Y. 1992. Glycoprotein pharmaceuticals—scientific and regulatory considerations, and the United-States Orphan Drug-Act. *Trends Biotechnol.* 10:114–120.
3. Abeijon, C. and Hirschberg, C.B. 1992. Topography of glycosylation reactions in the endoplasmic reticulum. *Trends Biochem. Sci.* 17:32–36.
4. Hayes, B.K. and Hart, G.W. 1994. Novel forms of protein glycosylation. *Curr. Opinion Struct. Biol.* 4:692–698.
5. Joziasse, D.H. 1992. Mammalian glycosyltransferases: genomic organization and protein structure. *Glycobiology* 2:271–277.
6. Field, M.C. and Wainwright, L.J. 1995. Molecular-cloning of eukaryotic glycoprotein and glycolipid glycosyltransferases—a survey. *Glycobiology* 5:483–472.
7. Varki, A. 1993. Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology* 3:97–130.
8. Paraikh, R.B. and Patel, T.R. 1992. Comparing the glycosylation patterns of recombinant glycoproteins. *Trends Biotechnol.* 10:276–280.
9. Jenkins, N. and Curling, E.M. 1994. Glycosylation of recombinant proteins: problems and prospects. *Enzyme Microb. Technol.* 16:354–364.
10. Goochee, C.F. and Monica, T.J. 1990. Environmental effects on protein glycosylation. *Bio/Technology* 8:421–427.
11. Goochee, C.F., Gramer, M.J., Andersen, D.C., Behr, J.B., and Rasmussen, J.R. 1991. The oligosaccharides of glycoproteins—bioprocess factors affecting oligosaccharide structure and their effect on glycoprotein properties. *Bio/Technology* 9:1347–1355.
12. Letourneur, O., Seoti, S., Willettebrown, J., Robertson, M.W., and Kinet, J.P. 1995. Glycosylation of human truncated Fc-epsilon-RI α-chain is necessary for efficient folding in the endoplasmic-reticulum. *J. Biol. Chem.* 270:8249–8256.
13. Stimson, E., Virji, M., Makepeace, K., Dell, A., Morris, H.R., Payne, G., et al. 1995. Meningococcal pilin—a glycoprotein substituted with digalactosyl 2,4-diacetamido-2,4,6-trideoxyhexose. *Mol. Microbiol.* 17:1201–1214.
14. Herscovics, A.O. and Orlean, P. 1993. Glycoprotein biosynthesis in yeast. *FASEB J.* 7:540–550.
15. Kniskern, P.J., Hagopian, A., Burke, P., Schutz, L.D., Montgomery, D.L., Hurni, W.M., et al. 1994. Characterization and evaluation of a recombinant hepatitis-B vaccine expressed in yeast defective for *N*-linked hyperglycosylation. *Vaccine* 12:1021–1025.
16. Lehle, L., Elden, A., Lehnert, K., Haselbeck, A., and Kopetzki, E. 1995. Glycoprotein-biosynthesis in *Saccharomyces cerevisiae*—ngd29, an *N*-glycosylation mutant allelic to och1 having a defect in the initiation of outer chain formation. *FEBS Lett.* 370:41–45.
17. Kalsner, I., Schneider, F.J., Geyet, R., Ahorn, H., and Maurerfogy, I. 1992. Comparison of the carbohydrate moieties of recombinant soluble Fc-epsilon receptor (sFc-ε-RII/cod23) expressed in *Saccharomyces cerevisiae* and chinese hamster ovary cells—different O-glycosylation sites are used by yeast and mammalian cells. *Glycoconjugate J.* 9:209–216.
18. Matsumoto, S., Ikura, K., Ueda, M., and Sasaki, R. 1995. Characterization of a human glycoprotein (erythropoietin) produced in cultured tobacco cells. *Plant Mol. Biol.* 27:1163–1172.
19. Altmann, F., Tretter, V., Kubelka, V., Staudacher, E., Marz, L., and Becker, W.M. 1993. Fucose in α1-3 linkage to the N-glycan core forms an allergenic epitope that occurs in plant and in insect glycoproteins. *Glycoconjugate J.* 10:301.
20. Ma, J.K.C. and Hein, M.B. 1995. Immunotherapeutic potential of antibodies produced in plants. *Trends Biotechnol.* 13:522–527.
21. James, D.C., Freedman, R.B., Hoare, M., Ogonah, O.W., Rooney, B.C., Larionov, O.A., et al. 1995. N-glycosylation of recombinant human interferon-γ produced in different animal expression systems. *Bio/Technology* 13:592–596.
22. Grabenhorst, E., Hofer, B., Nimtz, M., Jager, V., and Conradt, H.S. 1993. Biosynthesis and secretion of human interleukin-2 glycoprotein variants from baculovirus-infected sf21 cells—characterization of polypeptides and post-translational modifications. *Eur. J. Biochem.* 215:189–197.
23. Jarvis, D.L. and Finn, E.E. 1995. Biochemical-analysis of the N-glycosylation pathway in baculovirus-infected lepidopteran insect cells. *Virology* 212:500–511.
24. Davidson, D.J.C., Fraser, M.J., and Castellino, F.J. 1990. Oligosaccharide processing in the expression of human plasminogen cDNA by lepidopteran insect (*Spodoptera frugiperda*) cells. *Biochemistry* 29:5584–5590.
25. Ogonah, O.W., Freedman, R.B., Jenkins, N., Patel, K., and Rooney, B.C. 1996. Isolation and characterization of an insect-cell line able to perform complex N-linked glycosylation on recombinant proteins. *Bio/Technology* 14:197–202.
26. Davis, T.R. and Wood, H.A. 1995. Intrinsic glycosylation potentials of insect-cell cultures and insect larvae. *In Vitro Cellular Develop. Biol.-Animal* 31:659–663.
27. Larsen, R.D., Rivera-Marrero, C.A., Ernst, L.K., Cummings, R.D., and Lowe, J.B. 1990. Frameshift and nonsense mutations in a human genomic sequence homologous to a murine UDP-Gal:β-D-Gal(1,4)-D-GlcNAc α(1,3)-galactosyltransferase cDNA. *J. Biol. Chem.* 265:7055–7061.
28. Borrebaeck, C.A.K., Malmborg, A.C., and Ohlin, M. 1993. Does endogenous glycosylation prevent the use of mouse monoclonal antibodies as cancer therapeutics? *Immunol. Today* 14:477–479.
29. Tsuji, J., Noma, S., Suzuki, J., Okumura, K., and Shimizu, N. 1990. Specificity of human natural antibody to recombinant tissue-type plasminogen activator (t-PA) expressed in mouse C127 cells. *Chem. Pharm. Bull.* 38:765–768.
30. Lund, J.T., Takahashi, N., Hindley, S.A., Tyler, R., Goodall, M., and Jefferis, R. 1993. Glycosylation of human IgG subclass and mouse IgG2b heavy chains secreted by mouse J558L transfectoma cell lines as chimeric antibodies. *Human Antibodies & Hybridomas* 4:20–25.
31. Lund, J.T., Takahashi, N., Nakagawa, H., Goodall, M., Bentley, T., Hindley, S.A., et al. 1993. Control of IgG/Fc glycosylation: a comparison of oligosaccharides from chimeric human/mouse and mouse subclass immunoglobulin Gs. *Mol. Immunol.* 30:741–748.

32. Yu-Ip, C.C., Miller, W.J., Silberklang, M., Mark, G.E., Ellis, R.W., Huang, L.H., et al. 1994. Structural characterization of the N-glycans of a humanized anti-CD8 murine immunoglobulin G. *Arch. Biochem. Biophys.* 308:387–399.
33. Lifely, M.R., Hale, C., Boyce, S., Keen, M.J., and Phillips, J. 1995. Glycosylation and biological activity of CAMPATH-1H expressed in different cell lines and grown under different culture conditions. *Glycobiology* 5:813–822.
34. Stephens, S., Emtage, S., Vetterlein, O., Chaplin, L., Bebbington, C., Nesbitt, A., et al. 1995. Comprehensive pharmacokinetics of a humanized antibody and analysis of residual anti-idiotypic responses. *Immunology* 85:668–674.
35. Endo, T., Wright, A., Morrison, S.L., and Kobata, A. 1995. Glycosylation of the variable region of immunoglobulin G: site-specific maturation of the sugar chains. *Mol. Immunol.* 32:931–940.
36. Hamadeh, R.M., Jarvis, G.A., Galili, U., Mandrell, R.E., Zhou, P., and Griffiss, J.M. 1992. Human natural anti-Gal IgG regulates alternative complement pathway activation on bacterial surfaces. *J. Clin. Invest.* 89:1223–1235.
37. Lavecchio, J.A., Dunne, A.D., and Edge, A.S.B. 1995. Enzymatic removal of α-galactosyl epitopes from porcine endothelial cells diminishes the cytotoxic effect of natural antibodies. *Transplantation* 60:841–847.
38. Monica, T.J., Williams, S.B., Goochee, C.F., and Maiorella, B.L. 1995. Characterization of the glycosylation of a human-IgM produced by a human-mouse hybridoma. *Glycobiology* 5:175–185.
39. Muchmore, E.A., Milewski, M., Varki, A., and Diaz, S. 1989. Biosynthesis of N-glycolyneuraminic acid. The primary site of hydroxylation of N-acetylneuraminic acid is the cytosolic sugar nucleotide pool. *J. Biol. Chem.* 264:20216–20223.
40. Noguchi, A., Mukuria, C.J., Suzuki, E., and Naiki, M. 1995. Immunogenicity of N-glycolylneuraminic acid-containing carbohydrate chains of recombinant human erythropoietin expressed in chinese hamster ovary cells. *J. Biochem.* 117:59–62.
41. Flesher, A.R., Marzowski, J., Wang, W.C., and Raff, H.V. 1995. Fluorophore labeled glycan analysis of immunoglobulin fusion proteins: correlation of oligosaccharide content with in vivo clearance profile. *Biotechnol. Bioeng.* 46:399–407.
42. Kawano, T., Koyama, S., Takematsu, H., Kozutsumi, Y., Kawasaki, H., Kawashima, S., et al. 1995. Molecular cloning of cytidine monophospho-N-acetylneuraminic acid hydroxylase: regulation of species-specific and tissue-specific expression of N-glycolylneuraminic acid. *J. Biol. Chem.* 270:16458–16463.
43. Smith, D.F., Larsen, R.D., Mattox, S.A., Lowe, J.B., and Cummings, R.D. 1990. Transfer and expression of a murine UDP-Gal:β-D-Gal-α1,3-galactosyltransferase gene in transfected Chinese hamster ovary cells. Competition reactions between the α1,3-galactosyltransferase and the endogenous α2,3-sialyltransferase. *J. Biol. Chem.* 265:6225–6234.
44. Hokke, C.H., Bergwerff, A.A., Vandedem, G.W.K., Kamerling, J.P., and Vliegenthart, J.F.G. 1995. Structural-analysis of the sialylated N-linked and O-linked carbohydrate chains of recombinant-human-erythropoietin expressed in chinese-hamster ovary cells—sialylation patterns and branch location of dimeric N-acetyllactosamine units. *Eur. J. Biochem.* 228:981–1008.
45. Lee, E.U., Roth, J., and Paulson, J.C. 1989. Alteration of terminal glycosylation sequences on N-linked oligosaccharides of Chinese hamster ovary cells by expression of β-galactoside α2,6-sialyltransferase. *J. Biol. Chem.* 264:13848–13855.
46. Minch, S.L., Kallio, P.T., and Bailey, J.E. 1995. Tissue-plasminogen activator co-expressed in chinese-hamster ovary cells with α2,6-sialyltransferase contains NeuAc-α2,6gal-β1,4GlcNAc linkages. *Biotechnol. Prog.* 11:348–351.
47. Grabenhorst, E., Hoffmann, A., Nimtz, M., Zettlmeissl, G., and Conradt, H.S. 1995. Construction of stable BHK-21-cells coexpressing human secretory glycoproteins and human Gal(β-1-4)GlcNAc-r α-2,6-sialyltransferase α-2,6-linked NeuAc is preferentially attached to the Gal(β-1-4)GlcNAc(β-1-2)Man(α-1-3)-branch of diantennary oligosaccharides from secreted recombinant β-trace protein. *Eur. J. Biochem.* 232:718–725.
48. Stanley, P. and Ioffe, E. 1995. Glycosyltransferase mutants—key to new insights in glycobiology. *FASEB J.* 9:1436–1444.
49. Yamashita, K., Koida, N., Endo, T., Iwaki, Y., and Kobata, A. 1989. Altered glycosylation of serum transferrin of patients with hepatocellular carcinoma. *J. Biol. Chem.* 264:2415–2423.
50. Khan, M.W., Musgrave, S.C., and Jenkins, N. 1995. N-linked glycosylation of tissue-plasminogen activator in Namalwa cells. *Biochem. Soc. Trans.* 23:S99.
51. Okamoto, M., Nakai, M., Nakayama, C., Yanagi, H., Matsui, H., Noguchi, H., et al. 1991. Purification and characterization of three forms of differently glycosylated recombinant human granulocyte-macrophage colony-stimulating factor. *Arch. Biochem. Biophys.* 288:562–568.
52. Tandal, M., Endo, T., Sasaki, S., Masuho, Y., Kochiba, N., and Kobata, A. 1991. Structural study of the sugar moieties of monoclonal antibodies secreted by human-mouse hybridoma. *Arch. Biochem. Biophys.* 291:339–348.
53. Cole, E.S., Higgins, E., Bernasconi, R., Garone, L., and Edmunds, T. 1994. Glycosylation patterns of human proteins expressed in transgenic goat milk. *J. Cell Biochem.* 265:318D.
54. Prieto, P.A., Mukerji, P., Kelder, B., Erney, R., Gonzalez, D., Yun, J.S., et al. 1995. Remodeling of mouse milk glycoconjugates by transgenic expression of a human glycosyltransferase. *J. Biol. Chem.* 270:29515–29519.
55. Miyoshi, E., Ihara, Y., Hayashi, N., Fusamoto, H., Kamada, T., and Taniguchi, N. 1995. Transfection of N-acetylglucosaminyltransferase-III gene suppresses expression of hepatitis-B virus in a human hepatoma-cell line, HB611. *J. Biol. Chem.* 270:28311–28315.
56. Newkirk, M.M., Fournier, M.J., and Shirley, J. 1985. Rheumatoid-factor avidity in patients with rheumatoid-arthritis—identification of pathogenic RFs which correlate with disease parameters and with the Gal, glycoform of IgG. *J. Clin. Immunol.* 15:250–257.
57. Rademacher, T.W., Williams, P., and Dwek, R.A. 1994. Agalactosyl glycoforms of IgG autoantibodies are pathogenic. *Proc. Natl. Acad. Sci. USA* 91:6123–6127.
58. Sicilliano, R.A., Morris, H.R., Bennett, H.P.J., and Dell, A. 1994. O-glycosylation mimics N-glycosylation in the 16-kDa fragment of bovine pro-opiomelanocortin—the major O-glycan attached to Thr45 carries SO_4-4GalNAc-β1,4GlcNAc-β-1, which is the archetypal nonreducing epitope in the N-glycans of pituitary glycohormones. *J. Biol. Chem.* 269:910–920.
59. Bergwerff, A.A., Vanoostrum, J., Kamerling, J.P., and Vliegenthart, J.F.G. 1995. The major N-linked carbohydrate chains from human urokinase—the occurrence of 4-O-sulfated, (α2,6)-sialylated or (α-1,3)-fucosylated N-acetylgalactosamine(β-1-4)-N-acetylglucosamine elements. *Eur. J. Biochem.* 228:1009–1019.
60. Skelton, T.P., Kumar, S., Smith, P.L., Beranek, M.C., and Baenziger, J.U. 1992. Pro-opiomelanocortin synthesized by corticotrophs bears asparagine-linked oligosaccharides terminating with SO_4-4GalNAc β1,4GlcNAc-β1,2Man α. *J. Biol. Chem.* 267:12998–13008.
61. Smith, P.L. and Baenziger, J.U. 1992. Molecular basis of recognition by the glycoprotein hormone-specific N-acetylgalactosamine-transferase. *Proc. Natl. Acad. Sci. USA* 89:329–333.
62. Fiete, D., Srivastava, V., Hindsgaul, O., and Baenziger, J.U. 1991. A hepatic reticuloendothelial cell receptor specific for SO_4-4GalNAc-β1,4GlcNAc-β1,2Man-α that mediates rapid clearance of lutropin. *Cell* 67:1103–1110.
63. Pfeiffer, G., Strube, K.H., and Geyer, R. 1992. Biosynthesis of sulfated glycoprotein-N-glycans present in recombinant human tissue plasminogen activator. *Biochem. Biophys. Res. Commun.* 189:1681–1685.
64. Takei, Y., Chiba, T., Wada, K., Hayashi, H., Yamada, M., Kuwashima, J., et al. 1995. Glycosylated human recombinant interleukin-1-α, neo interleukin-1-α, with d-mannose dimer exhibits selective activities in-vivo. *J. Interferon & Cytokine Res.* 15:713–719.
65. Baudys, M., Uchio, T., Hovgaard, L., Zhu, E.F., Avramoglou, T., Jozefowicz, M., et al. 1995. Glycosylated insulins. *J. Controlled Release* 36:151–157.
66. Monroe, R.S. and Hubes, B.E. 1994. The major form of the murine asialoglycoprotein receptor—cDNA sequence and expression in liver, testis and epididymis. *Gene* 148:237–244.
67. Parekh, R.B., Dwek, R.A., Rademacher, T.W., Opdenakker, G., and Vandamme, J. 1992. Glycosylation of interleukin-6 purified from normal human blood mononuclear-cells. *Eur. J. Biochem.* 203:135–141.
68. Drickamer, K. 1991. Clearing up glycoprotein hormones. *Cell* 67:1029–1032.
69. Misaizu, T., Matsuki, S., Strickland, T.W., Takeuchi, M., Kobata, A., and Takasaki, S. 1995. Role of antennary structure of N-linked sugar chains in renal handling of recombinant human erythropoietin. *Blood* 86:4097–4104.
70. Speltman, M.W., Basa, L.J., Leonard, C.K., Chakel, J.A., O'Connor, J.V., Wilson, S. et al. 1989. Carbohydrate structures of human tissue plasminogen activator expressed in Chinese hamster ovary cells. *J. Biol. Chem.* 264:14100–14111.
71. Hotchkiss, A., Refino, C.J., Leonard, C.K., Oconnor, J.V., Crowley, C., Mccabe, J., et al. 1988. The influence of carbohydrate structure on the clearance of recombinant tissue-type plasminogen-activator. *Thromb. Haemost.* 60:255–261.
72. Patel, T.P., Parekh, R.B., Moellering, B.J., and Prior, C.P. 1992. Different culture methods lead to differences in glycosylation of a murine IgG monoclonal antibody. *Biochem. J.* 285:839–845.
73. Maiorella, B.L., Winkelhake, J., Young, J., Moyer, B., Bauer, R., Hora, M., et al. 1993. Effect of culture conditions on IgM antibody structure, pharmacokinetics and activity. *Bio/Technology* 11:387–392.
74. Gawlitzek, M., Valley, U., Nimtz, M., Wagner, R., and Conradt, H.S. 1995. Characterization of changes in the glycosylation pattern of recombinant proteins from BHK-21-cells due to different culture conditions. *J. Biotechnol.* 42:117–131.
75. Tachibana, H., Taniguchi, K., Ushio, Y., Teruya, K., Osada, K., and Murakami, H. 1994. Changes of monosaccharide availability of human hybridoma lead to alteration of biological properties of human monoclonal-antibody. *Cytotechnology* 16:151–157.
76. Hayter, P.M., Curling, E.M., Gould, M.L., Baines, A.J., Jenkins, N., Salmon, I., et al. 1993. The effect of dilution rate on CHO cell physiology and recombinant interferon-γ production in glucose-limited chemostat cultures. *Biotechnol. Bioeng.* 39:327–335.
77. Hayter, P.M., Curling, E.M., Baines, A.J., Jenkins, N., Salmon, I., Strange, P.G., et al. 1992. Glucose-limited chemostat culture of chinese hamster ovary cells producing recombinant human interferon-γ. *Biotechnol. Bioeng.* 39:327–335.
78. Castro, P.M.L., Ison, A.P., Hayter, P.M., and Bull, A.T. 1995. The macroheterogeneity of recombinant human interferon-γ produced by Chinese hamster ovary cells is affected by the protein and lipid content of the culture medium. *Biotechnol. Appl. Biochem.* 87:87–100.
79. Jenkins, N., Castro, P.M.L., Menon, S., Ison, A.P., and Bull, A.T. 1994. Effect of lipid supplements on the production and glycosylation of recombinant interferon-γ expressed in CHO cells. *Cytotechnology* 15:209–215.
80. Rijcken, W.R.P., Overdijk, B., Vandeneijnden, D.H., and Ferwerda, W. 1995. The effect of increasing nucleotide sugar concentrations on the incorporation of sugars into glycoconjugates in rat hepatocytes. *Biochem. J.* 305:865–870.

81. Robinson, D.K., Chan, C.P., Ip, C.Y., Tsai, P.K., Tung, J., Seamans, T.C., et al. 1994. Characterization of a recombinant antibody produced in the course of a high-yield fed-batch process. *Biotechnol. Bioeng.* 44:727–735.
82. Hooker, A.D., Goldman, M.H., Markham, N.H., James, D.C., Ison, A.P., Bull, A.T., et al. 1995. N-glycans of recombinant human interferon-γ change during batch culture of chinese-hamster ovary cells. *Biotechnol. Bioeng.* 46:639–648.
83. Gawlitzek, M., Conradt, H.S., and Wagner, R. 1995. Effect of different cell-culture conditions on the polypeptide integrity and N-glycosylation of a recombinant model glycoprotein. *Biotechnol. Bioeng.* 48:536–544.
84. Shelikoff, M., Sinskey, A.J., and Stephanopoulos, G. 1994. The effect of protein-synthesis inhibitors on the glycosylation site occupancy of recombinant human prolactin. *Cytotechnology* 15:195–208.
85. Bulleid, N.J., Bassel-Duby, R.S., Freedman, R.B., Sambrook, J.F., and Gething, M.J. 1992. Cell-free synthesis of enzymically active tissue-type plasminogen activator. Protein folding determines the extent of N-linked glycosylation. *Biochem. J.* 286:275–280.
86. Allen, S., Naim, H.Y., and Bulleid, N.J. 1995. Intracellular folding of tissue-type plasminogen-activator—effects of disulfide bond formation on N-linked glycosylation and secretion. *J. Biol. Chem.* 270:4797–4804.
87. Hahn, T.J. and Goochee, C.F. 1992. Growth-associated glycosylation of transferrin secreted by HepG2 cells. *J. Biol. Chem.* 267:23982–23987.
88. Datti, A. and Dennis, J.W. 1993. Regulation of UDP-GlcNAc:Gal β 1-3GalNAc-R β 1-6-N-acetylglucosaminyltransferase (GlcNAc to GalNAc) in Chinese hamster ovary cells. *J. Biol. Chem.* 268:5409–5416.
89. Chotigeat, W., Watanapokasin, Y., Mahler, S., and Gray, P.P. 1994. Role of environmental-conditions on the expression levels, glycoform pattern and levels of sialyltransferase for hFSH produced by recombinant CHO cells. *Cytotechnology* 15:217–221.
90. Gebert, C.A. and Gray, P.P. 1995. Expression of FSH in CHO cells .2. stimulation of hFSH expression levels by defined medium supplements. *Cytotechnology* 17:13–19.
91. Shah, S., Lance, P., Smith, T.J., Berenson, C.S., Cohen, S.A., Horvath, P.J., et al. 1992. N-butyrate reduces the expression of β-galactoside α2,6-sialyltransferase in Hep G2 cells. *J. Biol. Chem.* 267:10852–10858.
92. Lin, A.A., Kimura, R., and Miller, W.M. 1993. Production of tPA in recombinant CHO cells under oxygen-limited conditions. *Biotechnol. Bioeng.* 42:339–350.
93. Borys, M.C., Linzer, D.J.H., and Papoutsakis, E.T. 1993. Culture pH affects expression rates and glycosylation of recombinant mouse placental lactogen proteins by chinese hamster ovary (CHO) cells. *Bio/Technology* 11:720–724.
94. Andersen, D.C., Goochee, C.F., Cooper, G., and Weitzhandler, M. 1994. Monosaccharide and oligosaccharide analysis of isoelectric focusing-separated and blotted granulocyte-colony-stimulating factor glycoforms using high-pH anion-exchange chromatography with pulsed amperometric detection. *Glycobiology* 4:459–467.
95. Borys, M.C., Linzer, D.I.H., and Papoutsakis, E.T. 1994. Ammonia affects the glycosylation patterns of recombinant mouse placental lactogen-1 by chinese-hamster ovary cells in a pH-dependent manner. *Biotechnol. Bioeng.* 43:505–514.
96. Gramer, M.J. and Goochee, C.F. 1993. Glycosidase activities in chinese-hamster ovary cell lysate and cell-culture supernatant. *Biotechnol. Prog.* 9:356–373.
97. Gramer, M.J., Goochee, C.F., Chock, V., Brousseau, D.T., and Silwkowski, M.B. 1995. Removal of sialic acid from a glycoprotein in CHO cell supernatant by action of an extracellular CHO cell sialidase. *Bio/Technology* 13:692–698.
98. Warner, T.G., Chang, J., Ferrari, J., Harris, R., Mcnemey, T., Bennett, G., et al. 1993. Isolation and properties of a soluble sialidase from the culture fluid of chinese-hamster ovary cells. *Glycobiology* 3:455–463.
99. Ferrari, J., Harris, R., and Warner, T.G. 1994. Cloning and expression of a soluble sialidase from chinese-hamster ovary cells—sequence alignment similarities to bacterial sialidases. *Glycobiology* 4:387–373.
100. Gramer, M.J., Schaffer, D.V., Silwkowski, M.B., and Goochee, C.F. 1994. Purification and characterization of α-l-fucosidase from chinese-hamster ovary cell-culture supernatant. *Glycobiology* 4:611–616.
101. Gramer, M.J. and Goochee, C.F. 1994. Glycosidase activities of the 293 and NS0 cell-lines, and of an antibody-producing hybridoma cell-line. *Biotechnol. Bioeng.* 43:423–428.
102. Ackermann, M., Marx, U., and Jager, V. 1996. Influence of cell-derived and media-derived factors on the integrity of a human monoclonal-antibody after secretion into serum-free cell-culture supernatants. *Biotechnol. Bioeng.* 49:97–106.
103. Matsuo, I., Nakahara, Y., Ito, Y., Nukada, T., and Ogawa, T. 1995. Synthesis of a glycopeptide carrying a N-linked core pentasaccharide. *Bio-organic & Med. Chem.* 3:1455–1463.
104. Nakahara, Y., Shibayama, S., and Ogawa, T. 1996. Rationally designed syntheses of high-mannose and complex type undecasaccharides. *Carbohyd. Res.* 280:67–84.
105. Herrmann, G.F., Ichikawa, Y., Wandrey, C., Gasta, F.C.A., Paulson, J.C., and Wong, C.H. 1993. A new multienzyme system for a one-pot synthesis of sialyl oligosaccharides—combined use of β-galactosidase and α(2, 6)-sialyltransferase coupled with regeneration in situ of CMP-sialic acid. *Tetrahedron Lett.* 34:3091–3094.
106. Galili, U. and Anaraki, F. 1995. α-galactosyl (Gal1-α-1-3Gal-β-1-4GlcNAc-r) epitopes on human-cells—synthesis of the epitope on human red-cells by recombinant primate α1,3-galactosyltransferase expressed in *Escherichia coli*. *Glycobiology* 5:775–782.